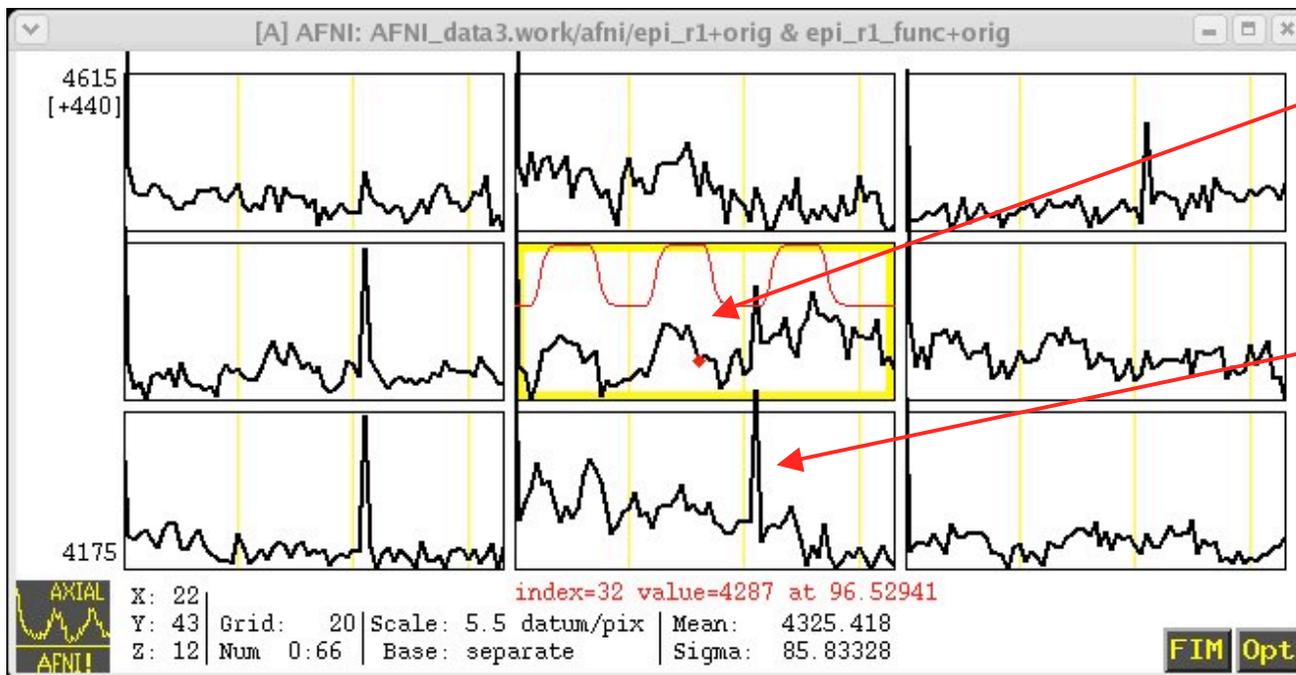


Sample Data Analysis: Simple Regression

- Enough theory (for now: more to come later!)
- To look at the data: type `cd AFNI_data3/afni` ; then `afni`
- **Switch Underlay** to dataset `epi_r1`
 - Then **Axial Image** and **Graph**
 - **FIM**→**Pick Ideal** ; then click `afni/epi_r1_ideal.1D` ; then **Set**
 - Right-click in image, **Jump to (ijk)**, then `22 43 12`, then **Set**



- Data clearly has activity in sync with reference
 - 30 s blocks
- Data also has a big spike in middle, which is annoying
 - Subject head movement!
 - Also spike at $t=0$

Preparing Data for Analysis

- Six preparatory steps are common:
 - Temporal alignment: program [3dTshift](#)
 - Image registration (AKA realignment): program [3dvolreg](#)
 - Image smoothing: program [3dmerge](#)
 - Image masking: [3dAutomask](#) or [3dClipLevel](#)
 - Conversion to percentile: programs [3dTstat](#) and [3dcalc](#)
 - Censoring out time points that are bad: program [3dToutcount](#) (or [3dTqual](#)) and [3dvolreg](#)
-
- Not all steps are necessary or desirable in any given case
 - In this first example, will only do registration, since the data obviously needs this correction

Data Analysis Script

- In file **epi_r1_regress**:

```
3dvolreg -base 3 \
        -verb \
        -prefix epi_r1_reg \
        -1Dfile epi_r1_mot.1D \
        epi_r1+orig
```

- **3dvolreg** (3D image registration) will be covered in detail in a later presentation
- filename to get estimated motion parameters

```
3dDeconvolve \
  -input epi_r1_reg+orig \
  -nfirst 3 \
  -num_stimts 1 \
  -stim_times 1 epi_r1_times.1D \
    'BLOCK(30) ' \
  -stim_label 1 AllStim \
  -tout \
  -bucket epi_r1_func \
  -fitts epi_r1_fitts \
  -xjpeg epi_r1_Xmat.jpg \
  -x1D epi_r1_Xmat.x1D
```

- **3dDeconvolve** = regression code
- Name of input dataset (from **3dvolreg**)
- Index of first sub-brick to process [skipping #0-2]
- Number of input model time series
- Name of input stimulus class timing file (τ 's) and type of HRF model to fit
- Name for results in AFNI menus
- Indicates to output t -statistic for β weights
- Name of output "bucket" dataset (statistics)
- Name of output model fit dataset
- Name of image file to store X [AKA R] matrix
- Name of text file in which to store X matrix

• Type **tcsh epi_r1_regress**; then wait for programs to run

Screen Output of the `epi_r1_decon` script

- 3dvolreg output

```

++ 3dvolreg: AFNI version=AFNI_2007_05_29_1644 (Sep  5 2007) [64-bit]
++ Reading input dataset ./epi_r1+orig.BRIK
++ Edging: x=3 y=3 z=2
++ Initializing alignment base
++ Starting final pass on 67 sub-bricks: 0..1..2..3.. *** ..63..64..65..66..
++ CPU time for realignment=5.35 s  [=0.0799 s/sub-brick]
++ Min  : roll=-0.103  pitch=-1.594  yaw=-0.038  dS=-0.354  dL=-0.021  dP=-0.191
++ Mean: roll=-0.047  pitch=+0.061  yaw=+0.023  dS=+0.006  dL=+0.032  dP=-0.076
++ Max  : roll=+0.065  pitch=+0.290  yaw=+0.055  dS=+0.050  dL=+0.120  dP=+0.113
++ Max displacement in automask = 2.46 (mm) at sub-brick 42 } Maximum movement estimate
++ Wrote dataset to disk in ./epi_r1_reg+orig.BRIK

```

- 3dDeconvolve output

```

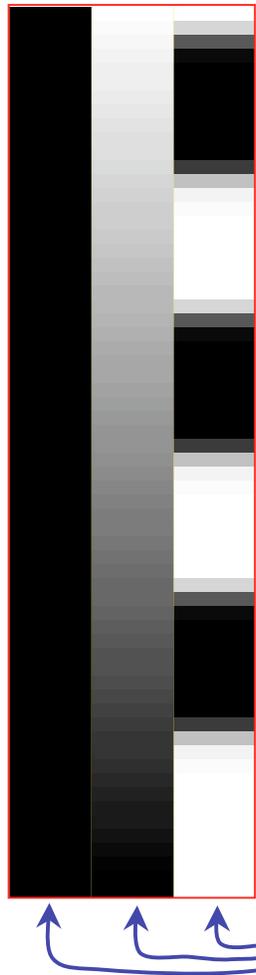
++3dDeconvolve: AFNI version=AFNI_2007_05_29_1644 (Sep  5 2007) [64-bit]
++ Authored by: B. Douglas Ward, et al.
++ loading dataset epi_r1_reg+orig
*+ WARNING: Input polort=1; Longest run=201.0 s; Recommended minimum polort=2 } Consider '-polort 2'
++ -stim_times using TR=3 seconds
++ '-stim_times 1' using LOCAL times
++ Wrote matrix image to file epi_r1_Xmat.jpg } Output file indicators
++ Wrote matrix values to file epi_r1_Xmat.x1D }
++ Signal+Baseline matrix condition [X] (64x3):  2.59165  ++ VERY GOOD ++
++ Signal-only matrix condition [X] (64x1):  1  ++ VERY GOOD ++
++ Baseline-only matrix condition [X] (64x2):  1.08449  ++ VERY GOOD ++
++ -polort-only matrix condition [X] (64x2):  1.08449  ++ VERY GOOD ++
++ Matrix inverse average error = 5.62791e-16  ++ VERY GOOD ++
++ Calculations starting; elapsed time=0.238
++ voxel loop:0123456789.0123456789.0123456789.0123456789.0123456789. } Progress meter / pacifier
++ Calculations finished; elapsed time=1.417
++ Wrote bucket dataset into ./epi_r1_func+orig.BRIK } Output file indicators
++ Wrote 3D+time dataset into ./epi_r1_fitts+orig.BRIK }
++ #Flops=3.11955e+08  Average Dot Product=4.50251

```

- If a program crashes, we'll need to see this text output (at the very least)!

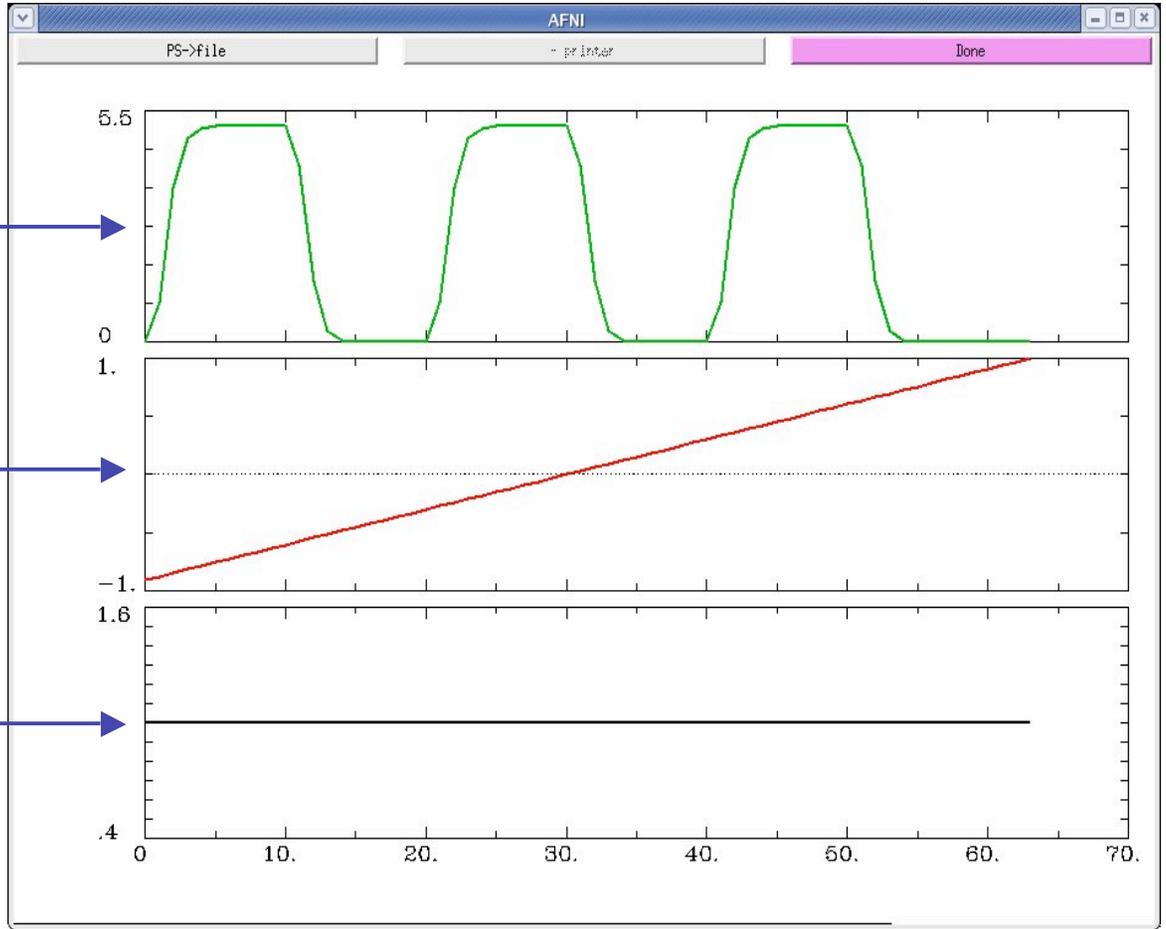
Stimulus Timing: Input and Visualization

`epi_r1_times.1D` = 9.0 69.0 129.0
= times of *start* of each BLOCK (30) HRF copy



X matrix columns

- HRF ⊗ timing →
- Linear in t →
- All ones →



`aiv epi_r1_Xmat.jpg`

`1dplot -sepscl epi_r1_Xmat.x1D`

Look at the Activation Map

- Run **afni** to view what we've got (N.B.: a weak test with only 1 run)
 - **Switch Underlay** to `epi_r1_reg` (input for **3dDeconvolve**)
 - **Switch Overlay** to `epi_r1_func` (output from **3dDeconvolve**)
 - **Sagittal Image** and **Graph** viewers
 - **FIM**→**Ignore**→**3** to have graph viewer not plot 1st 3 time pts
 - **FIM**→**Pick Ideal**; pick `epi_r1_ideal.1D` (modeled HRF: output from **waver**)
- **Define Overlay** to set up functional coloring
 - **Olay**→**Allstim#0_Coef** (sets coloring to be from model fit β)
 - **Thr**→**Allstim#0_Tstat** (sets threshold to be model fit t -statistic)
 - **See Overlay** (otherwise won't see the function!) – should be on automatically
 - Play with threshold slider to get a meaningful activation map (e.g., $t=3$ is a decent threshold — more on thresholds later)
 - Again, use **Jump to (i j k)** to jump to index coordinates **22 43 12**

More Looking at the Results

- Graph viewer: **Opt→Tran 1D→Dataset #N** to plot the model fit dataset output by **3dDeconvolve**
 - Will open the control panel for the **Dataset #N** plugin
 - Click first **Input** line to be 'on'; then choose **Dataset epi_r1_reg+orig**
 - Also choose **Color dk-blue** to get a pleasing plot
 - Click 2nd **Input** on; then choose **Dataset epi_r1_fitts+orig**
 - Also choose **Color limegreen** to get a pleasing plot
 - Then click on **Set+Close** (to close the plugin's control panel)
 - This tool lets you visualize the quality of the data fit
- Can also now overlay function on MP-RAGE anatomical by using **Switch Underlay** to **anat+orig** dataset
 - Probably won't want to graph the **anat+orig** dataset!

More Realistic Study

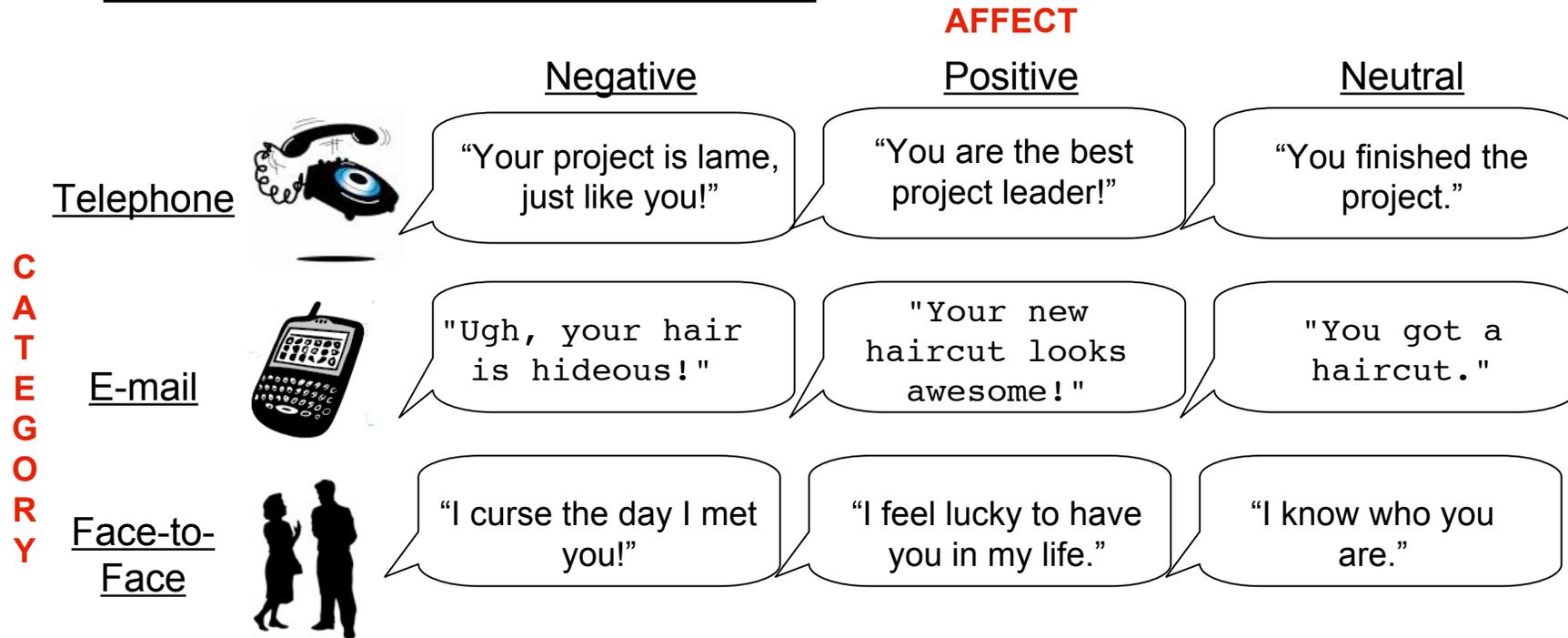
• The Experiment

- ★ Cognitive Task: Subjects see photographs of two people interacting
 - The mode of communication falls in one of 3 *categories*: via telephone, email, or face-to-face.
 - The *affect* portrayed is either negative, positive, or neutral in nature.

- ★ Experimental Design: 3x3 Factorial design, BLOCKED trials
 - Factor A: **CATEGORY** - (1) Telephone, (2) E-mail, (3) Face-to-Face
 - Factor B: **AFFECT** - (1) Negative, (2) Positive, (3) Neutral

 - A random 30-second block of photographs for a task (ON), followed by a 30-second block of the control condition of scrambled photographs (OFF)...
 - Each run has 3 ON blocks, 3 OFF blocks. 9 runs in a scanning session.

★ Illustration of Stimulus Conditions



★ Data Collected

- 1 Anatomical (MPRAGE) dataset for each subject
 - ➔ 124 axial slices
 - ➔ voxel dimensions = 0.938 x 0.938 x 1.2 mm
- 9 Time Series (EPI) datasets for each subject
 - ➔ 34 axial slices x 67 volumes = 2278 slices per run
 - ➔ TR = 3 sec; voxel dimensions = 3.75 x 3.75 x 3.5 mm
- Sample size, n=16 (all right handed)

Multiple Stimulus Classes

- Summary of the experiment
 - 9 related communication stimulus types in a 3x3 design of **Category** by **Affect** (stimuli are shown to subject as pictures)
 - **Telephone, Email & Face-to-face** = categories
 - **Negative, Positive & Neutral** = affects
 - ✓ telephone stimuli: **tneg, tpos, tneu**
 - ✓ email stimuli: **eneg, epos, eneu**
 - ✓ face-to-face stimuli: **fneg, fpos, fneu**
 - Each stimulus type has 3 presentation blocks of 30 s duration
 - Scrambled pictures (baseline) are shown between blocks
 - 9 imaging runs, 64 useful time points in each
 - Originally, 67 TRs per run, but skip first 3 for MRI signal to reach steady state (i.e., eliminate initial transient spike in data)
 - So 576 TRs of data, in total (64×9)
 - Already registered and put together into one dataset: **rall_vr+orig**

Regression with Multiple Model Files

- Script file **rall_decon** does the job:
- Run this script by typing **tcsh rall_regress** (takes a few minutes)

```
3dDeconvolve -input rall_vr+orig \
  -jobs 2 \ ← try to use 2 CPUs
  -concat '1D: 0 64 128 192 256 320 384 448 512' \ ← run start indexes
  -num_stimts 15 -local_times \
  -stim_times 1 '1D: 0 | | | 120 | | | | 60' 'BLOCK(30)' \ ← stimulus times
  -stim_times 2 '1D: * | | 120 | | 0 | | | 120' 'BLOCK(30)' \ ← 'l' indicates new run
  -stim_times 3 '1D: * | 120 | | 60 | | | | 0' 'BLOCK(30)' \ ← response model
  -stim_times 4 '1D: 60 | | | | 120 | 0 | |' 'BLOCK(30)' \
  -stim_times 5 '1D: * | 60 | | 0 | | | 120 | |' 'BLOCK(30)' \
  -stim_times 6 '1D: * | | 0 | | 60 | | | 60 |' 'BLOCK(30)' \
  -stim_times 7 '1D: * | 0 | | | 120 | | 60 | |' 'BLOCK(30)' \
  -stim_times 8 '1D: 120 | | | | 60 | | 0 |' 'BLOCK(30)' \
  -stim_times 9 '1D: * | | 60 | | | 0 | | 120 |' 'BLOCK(30)' \
  -stim_label 1 tneg -stim_label 2 tpos -stim_label 3 tneu \ ← stimulus label
  -stim_label 4 eneg -stim_label 5 epos -stim_label 6 eneu \
  -stim_label 7 fneg -stim_label 8 fpos -stim_label 9 fneu \
```

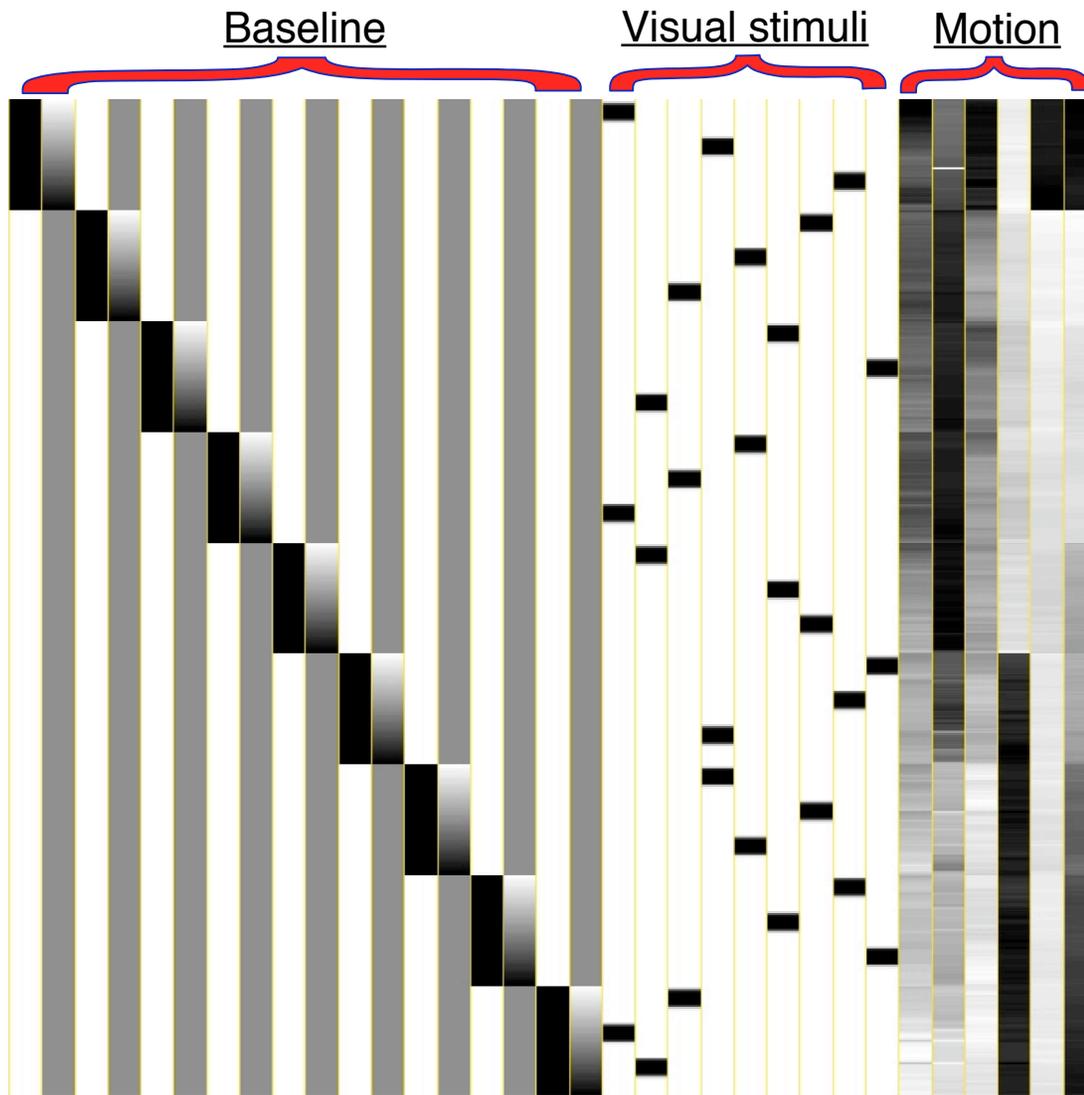
continued ...

Regression with Multiple Model Files (continued)

```
-stim_file 10 motion.1D'[0]' -stim_base 10      \ ← motion regressor
-stim_file 11 motion.1D'[1]' -stim_base 11      \ ← apply to baseline
-stim_file 12 motion.1D'[2]' -stim_base 12      \
-stim_file 13 motion.1D'[3]' -stim_base 13      \
-stim_file 14 motion.1D'[4]' -stim_base 14      \
-stim_file 15 motion.1D'[5]' -stim_base 15      \
-gltsym 'SYM: tpos -epos' -glt_label 1 TPvsEP    \ ← symbolic GLT
-gltsym 'SYM: tpos -tneg' -glt_label 2 TPvsTNg    \ ← label the GLT
-gltsym 'SYM: tpos tneu tneg -epos -eneu -eneg'   \
      -glt_label 3 TvseE                          \
-fout -tout                                       \ ← statistic types to output
-bucket rall_func -fitts rall_fitts             \
-xjpeg rall_xmat.jpg -x1D rall_xmat.x1D
```

- 9 visual stimulus classes were given using `-stim_times`
- **important to include motion parameters as regressors?**
 - this helps to exclude stimulus correlated motion artifacts
 - 6 motion parameters specified as covariates of no interest via `-stim_file` and `-stim_base`
 - `3dvolreg` has previously been run, with the `-1Dfile` option, which gave us file `motion.1D`
 - can test the significance of the inclusion with `-gltsym`
 - Switch from `-stim_base` to `-stim_label roll...`
 - Use `-gltsym 'SYM: roll \ pitch \yaw \dS \dL \dP'`

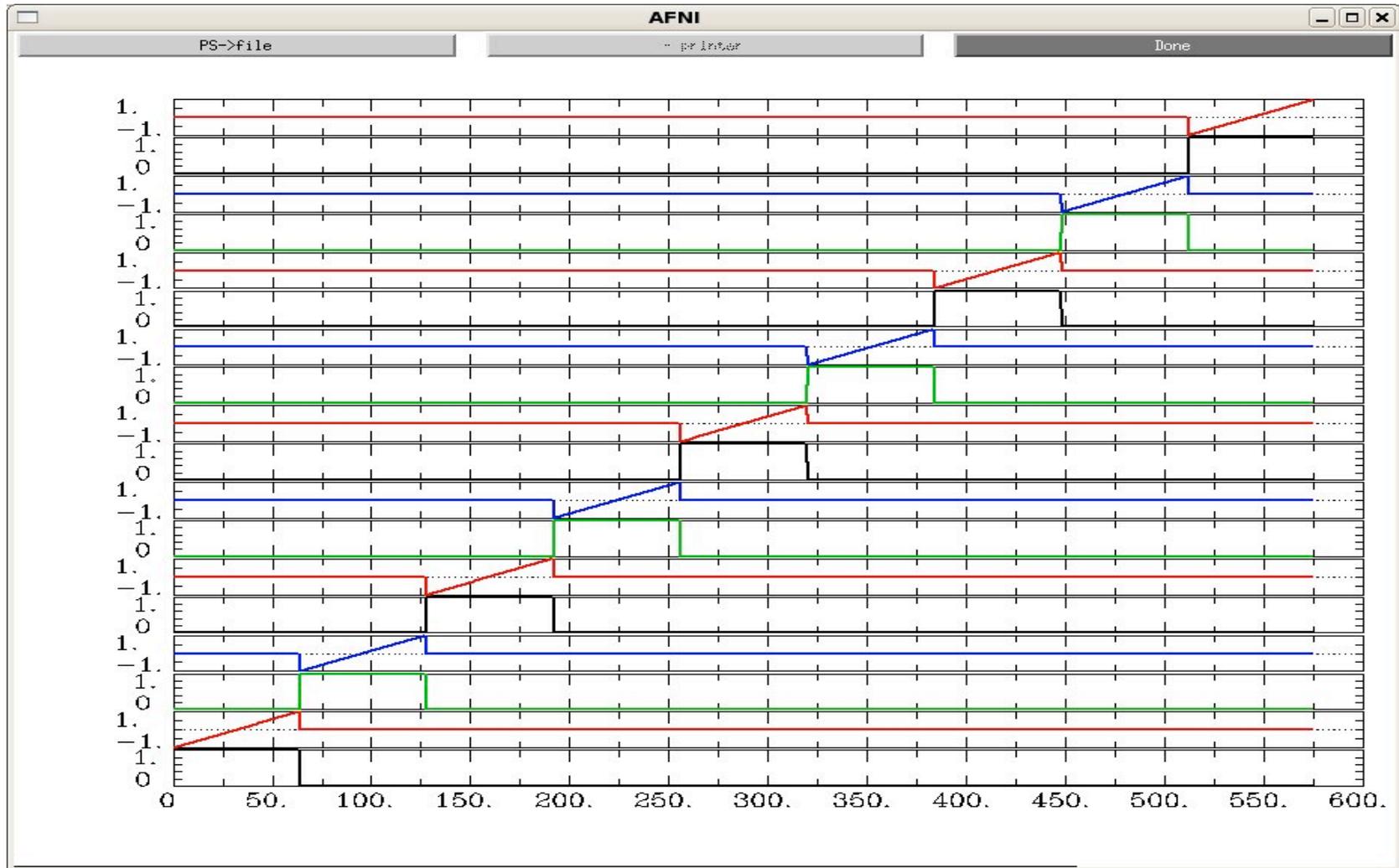
Regressor Matrix for This Script (via -xjpeg)



- 18 baseline regressors
 - linear baseline
 - 9 runs times 2 params
- 9 visual stimulus regressors
 - 3x3 design
- 6 motion regressors
 - 3 rotations and 3 shifts

`aiv_rall_xmat.jpg`

Regressor Matrix for This Script (via -x1D)

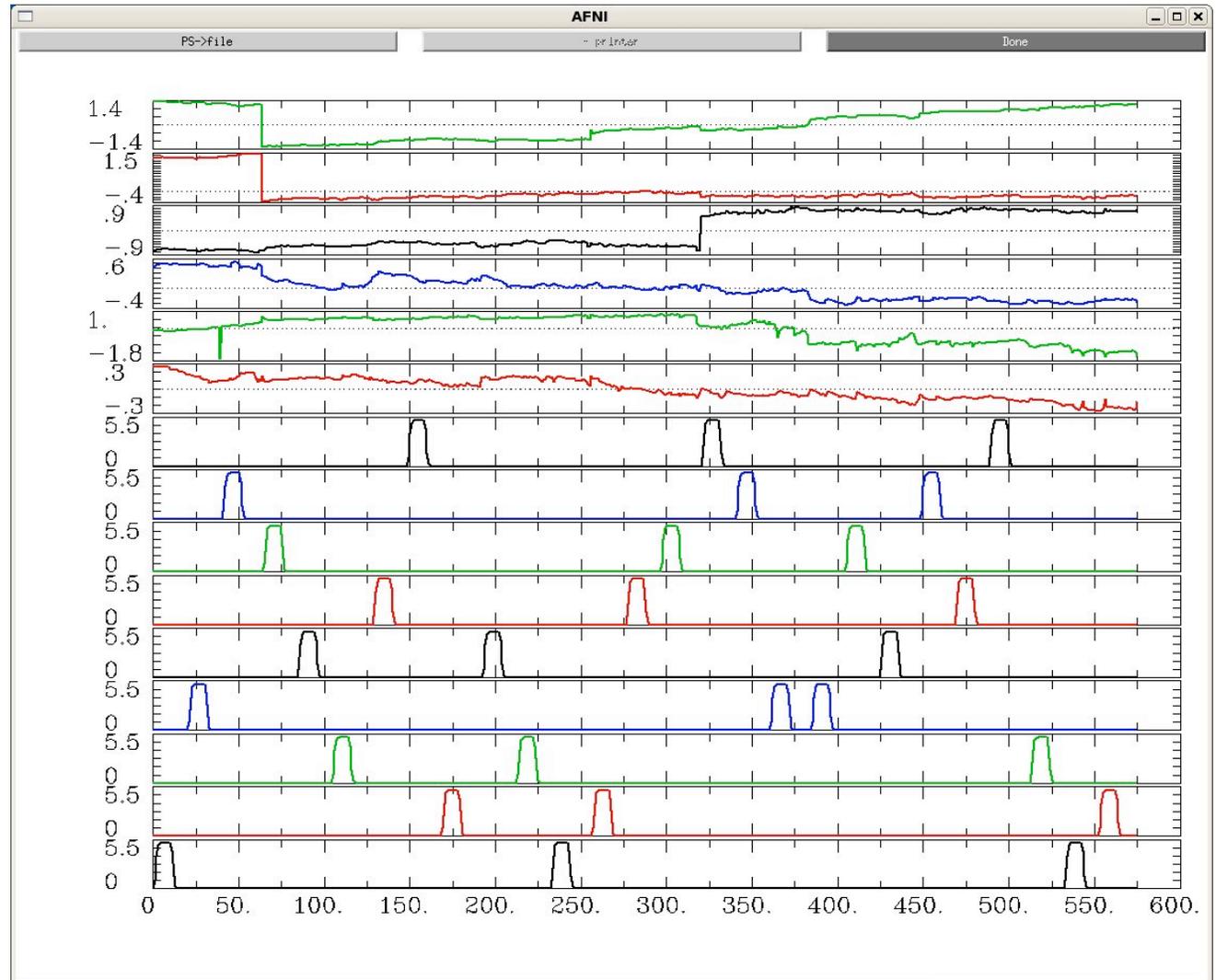


baseline regressors: via `1dplot -sepscl xmat_rall.x1D'[0..17]'`

Regressor Matrix for This Script (via -x1D)

- motion regressors

- visual stimuli



```
1dplot -sepscl xmat_rall.x1D'[18..$]'
```

Options in 3dDeconvolve - 1

```
-concat '1D: 0 64 128 192 256 320 384 448 512'
```

- “File” that indicates where distinct imaging runs start inside the input file
 - Numbers are the time indexes inside the dataset file for start of runs
 - In this case, a text format .1D file put directly on the command line
 - Could also be a filename, if you want to store that data externally

```
-num_stimts 15 -local_times
```

- We have 9 visual stimuli (+6 motion), so will need 9 `-stim_times` below
- Times given in the `-stim_times` files are *local* to the start of each run (vs. `-global_times` meaning times are relative the start of the first run)

```
-stim_times 1
```

```
'1D: 0.0 | | | 120.0 | | | | 60.0'  
'BLOCK(30)'
```

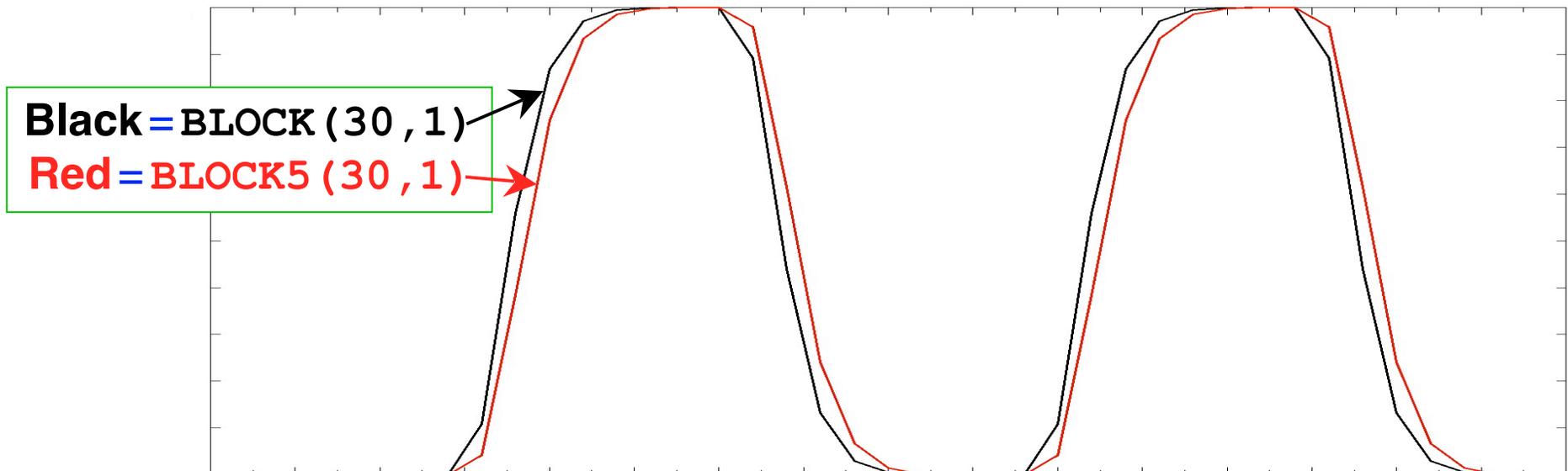
- “File” with 9 lines, each line specifying the start time in seconds for the stimuli within the corresponding imaging run, with the time measured relative to the start of the imaging run itself (local time)

Aside: the 'BLOCK ()' HRF Model

- **BLOCK (L)** is convolution of square wave of duration **L** with “gamma variate function” $t^4 e^{-t} / [4^4 e^{-4}]$ (peak value=1 at $t=4$):

$$h(t) = \int_0^{\min(t,L)} s^4 e^{-s} / [4^4 e^{-4}] ds$$

- “Hidden” option: **BLOCK5** replaces “4” with “5” in the above
 - Slightly more delayed rise and fall times
- **BLOCK (L, 1)** makes peak amplitude of *block* response = 1



Options in 3dDeconvolve - 2

```
-gltsym 'SYM: tpos -epos' -glt_label 1 TPvsEP
```

- **GLTs** are **General Linear Tests**
- **3dDeconvolve** provides test statistics for each regressor separately, but if you want to test combinations or contrasts of the β weights in each voxel, you need the **-gltsym** option
- Example above tests the difference between the β weights for the **Positive Telephone** and the **Positive Email** responses
 - Starting with **SYM:** means symbolic input is on command line
 - Otherwise inputs will be read from a file
 - Symbolic names for each regressor taken from **-stim_label** options
 - Stimulus label can be preceded by **+** or **-** to indicate sign to use in combination of β weights
 - **Leave space after each label!**
- Goal is to test a linear combination of the β weights
 - Tests if $\beta_{\text{tpos}} = \beta_{\text{epos}}$
 - e.g., does **tpos** get different response from **epos** ?
- Quiz: what would **'SYM: tpos epos'** test?

It would test if $\beta_{\text{tpos}} + \beta_{\text{epos}} = 0$

Options in 3dDeconvolve - 3

```
-gltsym 'SYM: tpos tneu tneg -epos -eneu -eneg'  
-glt_label 3 TvsE
```

- Goal is to test if $(\beta_{t_{\text{pos}}} + \beta_{t_{\text{neu}}} + \beta_{t_{\text{neg}}}) - (\beta_{e_{\text{pos}}} + \beta_{e_{\text{neu}}} + \beta_{e_{\text{neg}}}) = 0$
 - Test average BOLD signal change among the 3 affects in the telephone tasks versus the email tasks

```
-gltsym 'SYM: tpos -epos | tneu -eneu | tneg -eneg'  
-glt_label 3 TvsE_F
```

- Goal is to test if $\beta_{t_{\text{pos}}} = \beta_{e_{\text{pos}}}$, $\beta_{t_{\text{neu}}} = \beta_{e_{\text{neu}}}$, **and** $\beta_{t_{\text{neg}}} = \beta_{e_{\text{neg}}}$ are all true
 - BOLD signal change of any affect in the telephone tasks versus the email tasks
 - This is a different test than the previous one!
- `-glt_label 3 TvsE` option is used to attach a meaningful label to the resulting statistics sub-bricks
 - Output includes the ordered summation of the β weights and the associated statistical parameters (t - and/or F -statistics)
 - t - or F -statistics?

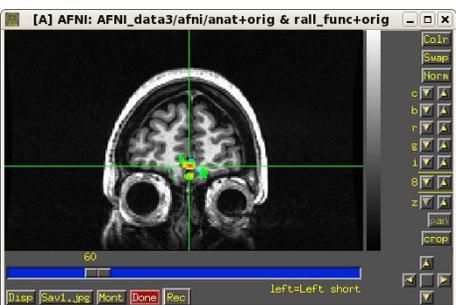
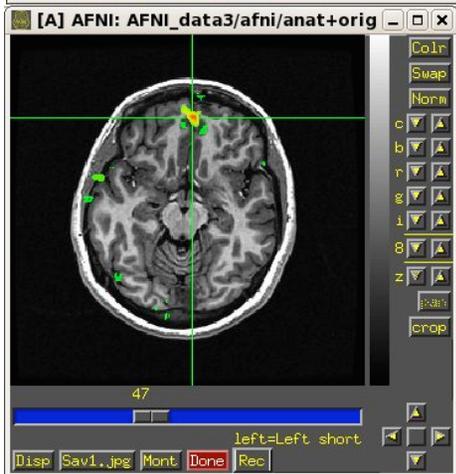
Options in 3dDeconvolve - 4

-fout -tout

= output both F - and t -statistics for each stimulus class ($-fout$) and stimulus coefficient ($-tout$) — but not for the baseline coefficients (if you want baseline statistics: $-bout$)

- The full model statistic is an F -statistic that shows how well the sum of all 9 input model time series fits voxel time series data
 - Compared to how well *just* the baseline model time series fit the data times (in this example, have 24 baseline regressor columns in the matrix — 18 for the linear baseline, plus 6 for motion regressors)
 - $F = [SSE(r) - SSE(f)] / df(n) \div [SSE(f) / df(d)]$
- The individual stimulus classes also will get individual F - and/or t -statistics indicating the significance of their individual *incremental* contributions to the data time series fit
 - e.g., F_{tpos} (#6, equivalent to t (#5)) tells if the full model explains more of the data variability than the model with t_{pos} omitted and all other model components included
 - If $DF=1$, t is equivalent to F : $t(n) = F^2(1, n)$

Results of **rall_regress** Script



```
# 0 Full_Fstat          #19 fneg#0_Coef
# 1 tneg#0_Coef        #20 fneg#0_Tstat
# 2 tneg#0_Tstat       #21 fneg_Fstat
# 3 tneg_Fstat         #22 fpos#0_Coef
# 4 tpos#0_Coef        #23 fpos#0_Tstat
# 5 tpos#0_Tstat       #24 fpos_Fstat
# 6 tpos_Fstat         #25 fneu#0_Coef
# 7 tneu#0_Coef        #26 fneu#0_Tstat
# 8 tneu#0_Tstat       #27 fneu_Fstat
# 9 tneu_Fstat         #28 TPvsEP_GLT#0_Coef
#10 eneg#0_Coef        #29 TPvsEP_GLT#0_Tstat
#11 eneg#0_Tstat       #30 TPvsEP_GLT_Fstat
#12 eneg_Fstat        #31 TPvsTNg_GLT#0_Coef
#13 epos#0_Coef        #32 TPvsTNg_GLT#0_Tstat
#14 epos#0_Tstat       #33 TPvsTNg_GLT_Fstat
#15 epos_Fstat        #34 TvsE_GLT#0_Coef
#16 eneu#0_Coef        #35 TvsE_GLT#0_Tstat
#17 eneu#0_Tstat       #36 TvsE_GLT_Fstat
#18 eneu_Fstat
```

• Images showing results from third GLT contrast: **TvsE**

• Menu showing labels from **3dDeconvolve** run

• Play with these results yourself!

Statistics from 3dDeconvolve

- An F -statistic measures significance of how much a model component (stimulus class) reduced the variance (sum of squares) of data time series residual
 - After all the other model components were given their chance to reduce the variance
 - **Residuals** \equiv data – model fit = errors = **-errts**
 - A t -statistic sub-brick measures impact of one coefficient (of course, **BLOCK** has only one coefficient)
- Full F measures how much the all regressors of interest combined reduced the variance over just the baseline regressors (**sub-brick #0**)
- Individual partial-model F s measures how much each individual signal regressor reduced data variance over the full model with that regressor excluded (e.g., **sub-bricks #3, #6, #9**)
- The **Coef** sub-bricks are the β weights (e.g., **#1, #4, #7, #10**) for the individual regressors
- Also present: GLT coefficients and statistics

# 0 Full_Fstat	#19 fneg#0_Coef
# 1 tneg#0_Coef	#20 fneg#0_Tstat
# 2 tneg#0_Tstat	#21 fneg_Fstat
# 3 tneg_Fstat	#22 fpos#0_Coef
# 4 tpos#0_Coef	#23 fpos#0_Tstat
# 5 tpos#0_Tstat	#24 fpos_Fstat
# 6 tpos_Fstat	#25 fneu#0_Coef
# 7 tneu#0_Coef	#26 fneu#0_Tstat
# 8 tneu#0_Tstat	#27 fneu_Fstat
# 9 tneu_Fstat	#28 TPvsEP_GLT#0_Coef
#10 eneg#0_Coef	#29 TPvsEP_GLT#0_Tstat
#11 eneg#0_Tstat	#30 TPvsEP_GLT_Fstat
#12 eneg_Fstat	#31 TPvsTNg_GLT#0_Coef
#13 epos#0_Coef	#32 TPvsTNg_GLT#0_Tstat
#14 epos#0_Tstat	#33 TPvsTNg_GLT_Fstat
#15 epos_Fstat	#34 TvsE_GLT#0_Coef
#16 eneu#0_Coef	#35 TvsE_GLT#0_Tstat
#17 eneu#0_Tstat	#36 TvsE_GLT_Fstat
#18 eneu_Fstat	

Group Analysis: will be carried out on β or **GLT** coefs from single-subject analyses